99. (New) RNA of about 21 to about 23 nucleotides produced by the method of Claim 96.

100. (New) A method of producing RNA of from about 21 to about 23 nucleotides in length that mediates RNA interference of mRNA of a gene to be degraded, comprising:

a) combining RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and

b) maintaining the combination of (a) under conditions under which the RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA.

101. (New) The method of Claim 100, wherein the soluble extract is derived from syncytial blastoderm Drosophila embryos.

102. (New) The method of Claim 100 further comprising isolating RNA of from about 21 to about 23 nucleotides from the combination.

REMARKS

Claim amendments

Claims 76-102 have been added.

Claims 76-85 relate to isolated double-stranded RNA of from about 21 to about 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds. Support for new Claims 76-85 can be found, for example, on page 14, line 21.

Claims 86-91 relate to isolated RNA of from about 21 to about 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds, wherein the isolated RNA is obtained from double-stranded RNA that has been cleaved into fragments of about 21 to about 23 nucleotides. Support for new Claims 86-91 can be found, for example, on page 15, lines line 26-28 and page 39, line 11 - page 40, line 7.

Claims 92-95 relate to isolated DNA comprising DNA encoding RNA of from about 21 to about 23 nucleotides that: mediate RNA interference of mRNA to which the RNA correspond; inactivate a corresponding gene by transcriptional silencing; mediate RNA interference of mRNA of a gene; and that target mRNA of a protein for degradation. Support for Claims 92-95 can be found, for example, on page 4, lines 8-16; page 5, lines 13-14 and 21-24; page 14, lines 9-10; page 15, lines 15-16; page 18, lines 9-12; and original Claims 5, 35 and 36.

Claims 96-99 relate to methods of producing RNA of from about 21 to about 23 nucleotides in length comprising combining RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and maintaining the combination under conditions in which the RNA is processed to RNA of from about 21 to about 23 nucleotides in length. Support for the claims can be found, for example, on page 14, lines 20-21 and in original Claims 9-12.

Claims 100-102 relate to methods producing RNA of from about 21 to about 23 nucleotides in length that mediates RNA interference of mRNA of a gene to be degraded, comprising combining RNA that corresponds to a sequence of the gene to be degraded with a soluble extract that mediates RNA interference, thereby producing a combination; and maintaining the combination under conditions under which the RNA is processed to RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA of the gene to be degraded, thereby producing RNA of from about 21 to about 23 nucleotides that mediates RNA interference of the mRNA. Support for Claims 100-102 can be found, for example, on page 14, lines 20-21 and in original Claims 13-15.

Third Supplemental Information Disclosure Statement

A Third Supplemental Information Disclosure Statement (IDS) is being filed concurrently. Entry of the Third IDS is respectfully requested.

Rejection of Claims 1-5, 12, 16, 48-50 and 72-75 under 35 U.S.C. §112, first paragraph

Claims 1-5, 12, 16, 48-50 and 72-75 are rejected under 35 U.S.C. §112, first paragraph "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention" (Office Action, page 3). The Examiner states that "applicants have expressed possession of only one species in a genus, which comprises hundreds of millions of different possibilities" (Office Action, page 3). The Examiner further states that "no common elements or attributes of the structural information (sequences) are disclosed" (Office Action, page 3).

Applicants respectfully disagree. Applicants disclose that "the common elements or attributes of the structural information" are that the claimed RNA is 1) about 21 to about 23 nt and 2) mediates RNAi of mRNA from which the 21 to 23 nt RNA corresponds. Applicants have disclosed ***two*** distinct RNA sequences of about 21 to about 23 nucleotides which mediate RNA interference of the mRNAs to which they correspond. Specifically, Applicants have shown that a 21 to 23 nt RNA fragment of Rr-dsRNA (Rr luciferase) and a 21 to 23 nt RNA fragment of Pp-dsRNA (Pp luciferase) mediate targeted destruction (*i.e.*, RNAi) of their respective mRNA (specification, Examples 1 and 2).

The Examiner further states that "[w]ith regard to the isolated RNA, it is insufficient to demonstrate identity of regulatory activity (mediates RNA interference) where no structural information regarding wherein in the RNA the activity resides"; that "no information is given regarding a methodology to determine such common elements or attributes"; and that "there is no description of variants"(Office Action, page 4). The Examiner cites Fiers v. Sugano in support of the rejection. The Examiner concludes that "at the time of filing, there is no record or description, which would demonstrate conception or written description of any structural information of isolated RNA or an analog of an isolated RNA or isolated DNA encoding said RNA with retaining correlative function in the claimed product" (Office Action, page 4).

Applicants again respectfully disagree. The Fiers v. Sugano case involved an interference among three parties over a count directed to a DNA which codes for a human fibroblast interferon-beta polypeptide. Priority was awarded to the only party (*i.e.*, Sugano) that set forth the complete and correct nucleotide sequence of a DNA encoding a human fibroblast interferon-beta polypeptide.

The court has recently discussed the written description requirement and clearly pointed out that:

> In its Guidelines, the PTO has determined that the written description requirement can be met by 'show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics' (Enzo Biochem, Inc., v. Gen-Probe 63 U.S.P.Q.2d 1609 (Fed. Cir., July 15, 2003)).

In the specification as filed, Applicants disclosed sufficiently detailed, relevant characteristics of the claimed RNA (*i.e.*, functional characteristics coupled with a known correlation between function and structure), and thus, have met the written description requirement.

Applicants do not claim a DNA which codes for a polypeptide as in the Fiers v. Sugano case. Rather, Applicants claim isolated RNA having a structural formula of from about 21 to about 23 nucleotides (nt) which functions to mediate RNA interference (RNAi) of the mRNA to which the 21 to 23 (nt) sequence corresponds. The function resides in the 21 to 23 nt RNA sequence which, for example, corresponds to the mRNA of a known target sequence. Applicants clearly teach in the specification that a molecular signal which recruits cellular factors involved in RNAi is likely present in these 21-23 nt fragments (specification, page 14, lines 5-7).

In particular, Applicants describe the development of an *in vitro* Drosophila embryo lysate that recapitulates double-stranded (ds) RNA-dependent silencing of gene expression (specification, page 20, lines 4-19; Example 1). Using this *in vitro* system, Applicants show that dsRNA incubated in the Drosophila lysate processes the dsRNA to a population of 21-23 nt RNA fragments (specification, page 20, line 20 - page 21, line 1; Example 2); that the 21-23 nt RNA fragments isolated from the incubation reaction caused sequence-specific RNAi (*i.e.*, caused RNAi of mRNA which corresponds to the dsRNA that was processed into 21-23 nt fragments in Applicants' *in vitro* system) (specification, Example 3); and that the 21-23 nt fragments purified by non-denaturing conditions from Applicants' *in vitro* system also caused sequence-specific RNAi (specification, Example 4). Applicants also show that the claimed fragments of the target sequence can be synthesized and used to mediate RNAi of the target mRNA sequence (specification, Example 5).

Thus, Applicants describe the structure (RNA from about 21 to about 23 nucleotides) and the function (mediate RNAi of an mRNA to which it corresponds) of the claimed isolated

RNA, methods for obtaining such fragments and methods for assessing whether such fragments mediate RNA interference of an mRNA to which the fragments correspond using 2 distinct, known genes (Rr luciferase and Pp luciferase). Applicants teach in the specification as filed that "[a]ny dsRNA can be used in the methods of the present invention, provided that it has sufficient homology to the targeted gene to mediate RNAi" (specification, page 17, lines 6-7). The sequences of numerous genes and polypeptides and their corresponding dsRNAs and mRNAs, are known to those of skill in the art.

In the specification as filed, Applicants have clearly described isolated RNA having a structural formula of from about 21 to about 23 nucleotides (nt) which functions to mediate RNA interference (RNAi) of the mRNA to which the 21 to 23 (nt) sequence corresponds, which can be obtained using Applicants' *in vitro* system to process dsRNAs which corresponds to the sequences into a population of 23-23 nt fragments or by synthesizing 21 - 23 nt fragments which correspond to the mRNA of the sequence. Furthermore, one of skill in the art would recognize that at the time of the invention Applicants had possession of an analog of the claimed isolated RNA, since the addition, deletion, substitution or alteration of one or more nucleotides in a 21-23 nt RNA sequence is well within the skill in the art. Applicants also describe how to assess whether the claimed isolated RNA and analogs thereof have the ability to mediate RNAi of an mRNA to which the fragments correspond.

Clearly, a person of skill in the art would recognize that the inventor had possession of isolated double-stranded RNA of from about 21 to about 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds and analogs thereof. At the time the application was filed, Applicants had possession of the claimed invention.

<u>Rejection of Claims 1-5, 12, 16 and 48-50 under 35 U.S.C.§102(b)</u>

Claims 1-5, 12, 16 and 48-50 are rejected under 35 U.S.C. §102(b) "as being anticipated" by Zamore *et al.* (Office Action, page 5). The Examiner states that Zamore *et al.* teach "translational regulation of hunchback mRNA"; "an isolated RNA of from about 21 to 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds"; that "shorter RNA having 22 nucleotides abolished (inactivates) the RNA binding activity (transcriptional activity)"; that "RNA comprises 3' UTR "; "chemical synthesis of RNA or analog of a naturally

occurring RNA"; that "analogs differs by addition of 5'-guanosine"; and "RNA isolation by non-denaturing gel electrophoresis and column chromatography" (Office Action, page 5).

Applicants respectfully disagree. As discussed in more detail below, Zamore *et al.* teach a sense strand of RNA which would not mediate RNA interference.

In particular, Zamore *et al.* describe "the in vitro binding affinity and stoichiometry" of ***a peptide (DmPUM-HD) for RNAs*** (Zamore *et al.*, page 603, column 1). Zamore *et al.* teach that "[t]ranslational repression of *hunchback* (*hb*) requires two copies of a bipartite sequence, the Nanos Response Element (NRE), located in the 3' untranslated region of the mRNA" and that the "PUMILIO (PUM) protein is thought to bind the NREs and thereby repress *hb* translation" (Zamore *et al.*, abstract). To analyze the binding affinity and stoichiometry of PUM, Zamore *et al.* expressed and purified the ***peptide***, dMPUM-HD, the active, soluble RNA-binding domain of the *Drosophila melanogaster* PUM and performed binding assays with the 181 RNA sequence, which includes the two copies of the NRE (*i.e.*, NRE1 and NRE2) in the context of the normal flanking sequences of the *hb* 3' UTR, and shorter versions of the 181 RNA sequence (Zamore *et al.*, Figures 2A-2C). Zamore *et al.* found that with the 181 RNA sequence, "one DmPUM-HD monomer binds independently and with equal affinity to each NRE" (Zamore *et al.*, abstract), however, the "shorter RNAs either reduced (37, 30, and 26 nt RNAs) or abolished (22 nt RNA) RNA binding" (Zamore *et al.*, page 600, column 2). Specifically, Zamore *et al.* show that the ***peptide***, dMPUM-HD, does not bind to an ***RNA*** of 22 nt of the NRE1 (Zamore *et al.*, Figure 2A). Furthermore, as pointed out above, Zamore *et al.* teach a sense strand of RNA which would not mediate RNA interference. Zamore *et al.* teach that the short RNAs "were transcribed from single-stranded oligonucleotide templates" (Zamore *et al.*, page 598, column 1). Clearly, Zamore *et al.* do not show an isolated RNA of from about 21 to 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds.

Zamore *et al.* do not anticipate Applicants' claimed invention, particularly as amended.

Rejection of Claims 72-75 under 35 U.S.C. §103(a)

Claims 72-75 are rejected under 35 U.S.C. §103(a) "as being unpatentable" over Zamore *et al.* in view of Wassenegger *et al.* (Office Action, page 6). The Examiner states that Zamore *et al.* "teach translational regulation of hunchback RNA" and "an isolated RNA of from about 21 to

23 nucleotides that mediates RNA interference of an mRNA to which it corresponds" (Office Action, page 6). The Examiner notes that Zamore *et al.* "did not teach a DNA encoding said RNA processed in eukaryotic cells (expression vector)" (Office Action, page 6). The Examiner cites Wassenegger *et al.* as teaching "nucleic acid molecules encoding coding region (mRNA) of RNA-directed RNA polymerase or encoding an enzymatically active fragment" and "transcriptional expression of said nucleic acid molecule in eukaryotic cells" (Office Action, pages 6-7). It is the Examiner's contention that:

> it would have been prima facie obvious to a person of skill in the art at the time the invention was made, to combine the transcriptional regulator RNA as taught by Zamore et al. with the expression system as taught by Wassenegger et al. to achieve expected advantage of developing an efficient expression vector . . . An ordinary practitioner would have been motivated to combine teachings of Zamore et al. with the expression system of Wassenegger et al. to achieve wide use of the isolated RNA molecules by incorporating the expression system because these limitations would improve the characterization of the nucleic acids (Office Action, page 7).

Applicants respectfully disagree. Where the claimed invention is rejected as obvious in view of a combination of references, § 103 requires both (1) that "the prior art would have suggested to the person of ordinary skill in the art that they should . . . carry out the claimed process"; and (2) that the prior art should establish a reasonable expectation of success (*In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991)). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *Id.* There is no teaching in the art cited directing the skilled person to isolate DNA encoding RNA that is processed in eukaryotic cells to RNA segments of about 21 to about 23 nucleotides in length that mediate RNA interference of mRNA to which the segments correspond.

As pointed out above, Zamore *et al.* teach a sense strand of RNA which would not mediate RNA interference. Zamore *et al.* do not teach or even suggest an isolated RNA of from about 21 to 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds. Zamore *et al.* teach the "in vitro binding affinity and stoichiometry" of ***DmPUM-HD, a peptide***, which binds to a 181 nt region in the 3' UTR of the *hb* ***mRNA***, thereby repressing *hb* translation. Zamore *et al.* show that shorter RNAs "either reduced (37, 30, and 26 nt RNAs) or abolished (22 nt RNA) RNA binding" by the DmPUM-HD ***peptide*** (Zamore *et al.*, page 600, column 2).

Wassenegger *et al.* used "a novel purification method for the isolation of a polypeptide having the enzymatic activity of an [RNA directed RNA polymerase] RdRP which was suitable for amino acid sequencing" (Wassenegger *et al.*, column 2, lines 63-66). Wassenegger *et al.* do not teach or even suggest an isolated double-stranded RNA of from about 21 to 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds.

There is no motivation to combine the teachings of the Zamore *et al.* and Wassenegger *et al.* references. Even if one of skill in the art were to make the improper combination, it would not render obvious Applicants' claimed invention, since using the expression system of Wassenegger *et al.* to express the transcriptional regulator RNA of Zamore *et al.* would not have produced isolated RNA of from about 21 to 23 nucleotides that mediates RNA interference of an mRNA to which it corresponds to the person of ordinary skill in the art.

Clearly, the combined teachings of Zamore *et al.* and Wassenegger *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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